Chapter I

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1.1. General

The history of the use of medicinal plants for the alleviation of diseases by human beings has its origin in primitive times. The plant kingdom contributed immensely to human health when no synthetic medicines were available, and when no concept of surgery existed. Primitive men lived in harmony with their surrounding environment. This entailed the use of herbal medicines as healthcare and also as medicinal treatment to various diseases. Illness, physical discomfort, wounds, and fear of death forced primitive men to use the natural substances around them. During antiquity, although guided by different cultural contexts, health in the Western and Eastern systems was based on holism, that is, treatment should act on the organism as a whole, integrated with the Universe. According to past records, Babylonians (about 3,000 B.C.) were aware of a large number of medicinal plants and their properties. Some of the plants are still used today in the same way and for the same purposes. The earliest mention of the medicinal use of plants in the Indian subcontinent is found in the Rig Veda (4,500–1,600 B.C.), which noted that the Indo-Aryans used the Soma plant (Amanita muscaria), a narcotic and hallucinogenic mushroom, as a medicinal agent.

1.2. World trend

Even with the progress of civilization men have not been able to dissociate themselves from plants rather the dependence is on a upward trend. The World Health Organization (WHO) has listed 20,000 medicinal plants globally (Gupta and Chadha, 1995). According to the WHO estimate, about 80 percent of the population in the
developing countries depends directly on plants for medicines (Pareek, 1996). There is a growing demand today for plant-based medicines, health products, pharmaceuticals, food supplements etc in the international market. The international market of medicinal plants is over 60 billion US dollar per year, which is growing at a rate of 7 percent per annum (Chatterjee, 2002). Medicinal, aromatic and dye plants (MADPs) also have potential to present as commodities with competitive advantages for the poor regions of Asia, Africa and Latin America. It is observed that some of the poorest regions of the world such as the Western and Eastern regions of the Himalayas, Borneo and Sumatra regions in Asia, Congo basin in Africa and Amazon catchments in South America are also rich in biological diversity where medicinal plants and other non-timber forest product (NTFP) species grow in abundance. If a balanced conservation and cultivation of medicinal, aromatic and dye plants can be promoted, the effort will protect human health, help to treat domestic animals to alleviate rural poverty, to address gender imbalances and to improve local economy.

1.3. Danger of extinction

Plant resources are depleting at an alarming rate and a number of economically and medicinally important plant species are likely to be extinct soon. An estimated 4,000-10,000 species of medicinal plants face potential local, regional or global extinction, with subsequent serious consequences for livelihoods, economies and healthcare systems (Hamilton, 2004). There are many potential causes of rarity in medicinal plant species, such as habitat specificity, narrow range of distribution, land use disturbances, introduction of non-natives, habitat alteration, climatic changes, heavy livestock grazing, explosion of human population, fragmentation and degradation of population, population
bottleneck and genetic drift. Additionally, natural enemies such as pathogens, herbivores and seed predators could substantially limit the abundance of rare medicinal plant species in any given area (Kala et al., 2006). Medicinal plants are now under great pressure due to their excessive collection or exploitation.

1.4. Indian scenario

India (80-30°N and 68-97.5°E) exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. This subcontinent is one of the world’s 12 leading biodiversity centres, encompassing 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and about 426 habitats of specific species. In India, more than 80 percent of medicinal and aromatic plants are collected from 17 million hectares of Indian forestland. There are about 17,000 species of higher plants, and 7,500 of them are known for medicinal uses (Kala et al., 2006). About 2,000 drugs used in the country are of plant origin (Dikshit, 1999).

With the increased collection, the degree of threat to natural populations of medicinal plants has increased because more than 90 percent of medicinal plant-raw materials for herbal industries in India and also for export is drawn from natural habitats (Dhar et al., 2002). At present, India exports herbal raw materials and medicines of about 100-114 million US dollar per year (Chatterjee, 2002). India and China are two of the largest countries in Asia, which have the richest arrays of registered and relatively well known medicinal plants (Raven, 1998). China is estimated to have 12,807 species of plants, out of which 11,146 are classified as medicinal plants used in traditional Chinese medicine (TCM). It is estimated that up to 492 species are currently under cultivation and the remaining 10,654 species are harvested from wild habitats (SEPA, 1997). According
to Wang *et al.* (2002), the total production of medicinal plants from the wild sources is 8.5 million tons and the production of cultivated medicinal plants was estimated to be 0.3 million tons in 2001-02. The plant based medicines not only contribute to the health of Chinese people but also add approximately 2 billion USD to the national economy annually.

Since, Indian subcontinent is well known for its diversity of forest products and the age-old healthcare traditions, there is an urgent need to establish these traditional values in both national and international perspectives realizing the ongoing developmental trends in traditional knowledge. A great deal of traditional knowledge on the use of various plant species is still intact with the indigenous people living in less accessible mountainous areas. The Planning Commission and the National Medicinal Plants Board (NMPB) of the Government of India have prepared a policy document on the promotional and commercial aspects of the medicinal plant sector. The Biodiversity Act 2002 framed many rules for the sustainable utilization of medicinal plants (Pus pangadan, 2005).

1.5. **Cultural preference**

Apart from human use, many plant species are used in animal husbandry as the primary source of healthcare in India. The reliance on medicinal plants is also due to the cultural preference of different communities. Medicinal plants have strong acceptance in religious activities of the native communities of India, who worship plants in the form of gods, goddesses and minor deities. *Origanum vulgare* (Oregano), *Saussurea obvallata* (snow lotus or Brahma Kamal), *Ocimum sanctum* (Holy Basil or Tulsi), *Cedrus deodara* (Cedarwood or Deodar cedar), *Cynodon dactylon* (Bermudagrass or Dubori), *Aegle*
*marmelos* (Bael fruit), *Juniperus communis* (Common juniper), *Nardostachys grandiflora* (Jatamansi or Indian Spikenard), *Zanthoxylum armatum* (Winged prickly-ash or Darmar), *Ficus benghalensis* (Indian Banyan tree) and *Ficus religiosa* (Bodhi tree) are some of the examples of plants highly used for medicinal as well as for religious purposes by the Hindus in Northern India (Kala *et al.*, 2006). The Buddhist community in Northern India regards *Terminalia chebula* (Myrobalan) as an important medicinal as well as a sacred fruit tree.

### 1.6. Alternative medicine

Already institutionalised to a large extent globally, alternative medicine forms a link between folk medicine and modern Western medicine. Many of the healing concepts date back to well before the advent of modern medicine. With the rapid and enormous progress of scientifically based modern Western medicines, they were however largely repressed. The last couple of decades have, notwithstanding, seen a global resurgence of these alternative medicinal systems, some of the best known being Homeopathy, Ayurvedic, Unani and traditional Chinese medicine.

There are two ancient systems of medicine in India, the Siddha that flourished in the South and the Ayurveda prevalent in the North. Ayurveda has alone reported approximately 2,000 medicinal plant species whereas more than 1,100 medicinal plant species are reported by Siddha (Kala *et al.*, 2006). The ‘Charak Samhita’, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs and their indigenous uses (Prajapati *et al.*, 2003). The traditional medical systems of India comprise of a major part of time-tested culture and are honoured by people till today.
These traditions have successfully set an example of natural resource utilization in curing many complex diseases for more than 3,000 years.

1.7. Merits of herbal therapy

Many advantages of such eco-friendly traditions exist. The plants used for various therapies are readily available, are easy to transport and have a relatively long shelf life. The most important advantage of herbal medicine is the minimal side effect and relatively low cost as compared to synthetic medicines. Synthetic drugs gained popularity against green remedies for their fast-acting effects; on the other hand, the healing process using medicinal plants is slow. But, people have begun to realize the benefits associated with natural remedies again. Chemically prepared drugs may act quickly but they have various side effects, which affect our body negatively in the long run, whereas medicinal plants work in an integrated or pro-biotic approach and with little adverse effects. For example, a regular intake of garlic can control cholesterol and high blood pressure within a moderate period of time, but taking synthetic drugs make the person’s body completely dependent on that particular medicine (Babu and Madhavi, 2001). Allopathic medicines may cure a wide range of diseases; however, its high prices and side effects are causing many people to return to herbal medicines.

1.8. Limitations of herbal medications

Apart from the slow healing process by the medicinal plants, another limitation of the herbal medication is that the actual working principles of most of the medications are still not known. Of late, a number of screening programmes, aimed at finding out new compounds of pharmacological activity or new sources of known compounds are being conducted. The instant increasing demand of plant-based drugs is unfortunately creating
heavy pressure on some selected high-value medicinal plant species in the wild due to over-harvesting. Several of these medicinal plant species possess slow growth, low population density and narrow geographic ranges; therefore, they are more prone to extinction (Kala et al., 2006).

1.9. North East India- a store house of potential medicinal plants

The North-East India is located between $87^032'\text{E} - 95^052'\text{E}$ and $21^034'\text{N} - 29^050'\text{N}$ and is a genetic treasure house of plant, animal and microbial resources. The region forms a distinctive part of the Indo-Burma hotspot which ranks the sixth among the 25 biodiversity hotspots of the world and is the prime one among the two identified in the Indian subcontinent (Sing, 2006). The region also falls in the bio-geographic tri-junction of Indian, the Himalayan and the oriental landmasses. The flora of the North Eastern Region is among the richest in the world and naturally a sizeable number of medicinal plants of tropical and subtropical nature grow here. The North Eastern Region of India that comprises of seven sister states viz. Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura along with Sikkim harbors more than 130 major tribal communities of the total of 427 tribal communities found in India (Sajem and Gosai, 2006). The North East India is blessed with nature’s bounty in regard to the wealth of industrially potential plants. The people from the region traditionally use various plant species as a home remedy for various ailments. But, proper phytochemical analysis of these plants is yet to be carried out. Modernization of the traditional knowledge and sustainable use of the medicinal plants through biochemical and biotechnological interventions will ensure the economic prosperity of the region. Some
important plant based medications prevalent among the different communities of North
Eastern states are given below:

*Alternanthera sessilis* R. Br. (Matikanduri, family- Amaranthaceae) is useful in diarrhoea
and dysentery

*Amaranthus spinosus* Linin (Huti khutura, family- Amaranthaceae) is used in leprosy,
bronchitis and fever.

*Andrographis paniculata* Burm. f Wall ex. Nees (Kalmegh, family- Acanthaceae) is
useful in fever, inflammations, cough and intestinal worms.

*Bacopa monieri* (L) Wettst (Brahmi, family- Scrophulariaceae) is used as a tonic for
nerves.

*Centella asiatica* (L) Urban (Bormanimuni, family- Umbelliferae) is useful in dysentery,
diarrhoea and skin diseases.

*Clerodendron colebrookianum* Walp (Nephaphu, family- Verbenaceae) leaf is used in
high blood pressure

*Cajanus cajan* (L) Mill (Rohar mah, family- Leguminosae) leaf is consumed to cure
jaundice.

*Drymaria cordata* Willd (Lai jabori, family- Caryophyllaceae) is useful in sinus
problems.

*Euphorbia hirta* Linn. (Dudh bon, family- Euphorbiaceae) is used to cure bronchitis.

*Flemingia strobilifera* R.Br (Makhti, family- Fabaceae) is used against dental
bleeding.

*Jatropha curcas* Linn. (Bongali era or bhut era, family- Euphorbiaceae) is used to cure
toothache.
*Meyna laxiflora* Robyns. (Khutura, family- Rubiaceae) is useful for piles and dysentery. *Rauvolfia serpentina* (L) Benth ex. Kurg (Sarpagandha, family- Apocynaceae) roots are advocated for high blood pressure.

*Stellaria media* Linn. (Morolia sak, family- Caryophyllaceae) is useful against enlargement of spleen and ulcers. *Withania somnifera* Dunal (Aswagandha, family- Solanaceae) is useful for rheumatism and nervous disorder.

1.10. Medicinal flora of Assam

Assam is specially renowned for its herbal treasure. Dependence on traditional herbs has a long history in Assam. The flora of the state is among the richest in the world and a number of classical medicinal plants and other high value plants of tropical and sub-tropical nature grow here. It has been estimated that there are about 3,000 species of plants having medicinal properties to cure and prevent different diseases (Kalita, 2006). The rural areas of the state inhabited by different communities particularly by the tribal people and socio-economically backward communities are the major sources of traditional knowledge about the ethnomedicinal use of the medicinal plants. The village elders as well as ‘Kabiraj’, ‘Ozas’ or ‘Bezs’ have traditionally used these medicinal plants to cure different ailments. They prepare medicines from different parts of medicinal plants. Various communities of Assam have their own traditional plant-based medications for the treatment.

Assam has a geographical area of 78,563 sq. km. with an average annual rainfall of 2,500 mm. The state is divided into six agroclimatic zones viz. North Bank Plains, Upper Brahmaputra Valley, Lower Brahmaputra Valley, Central Brahmaputra Valley,
Barak Valley and Hill Zone. The state has a sizable forest area. This includes reserved forests including national parks, biosphere reserves and sanctuaries, unclassed forests, village forests, private forests and botanical gardens. They harbor very rich species diversity. Although the state is very rich in medicinal plant diversity, the exploration is not up to the mark. The continuous increase in the demand and expanding trade on medicinal and aromatic plants worldwide, have jeopardized the survival of several plant species due to indiscriminate harvesting of natural flora including those in forests. It was estimated that about 90% collection of medicinal plants is from wild source and 70% of collections involve destructive harvesting. As a result of that, many species become extinct and some are endangered. For the sustainable growth in this sector, some necessary steps have to be initiated. Firstly, necessary legislative, legal and administrative legislations have to be made to prohibit the collection of those wild plant species which are rare, threatened, endangered and vulnerable. Secondly, the indigenous knowledge system has to be well documented. The traditional medicinal (herbal) practitioners should be given due recognition, their efforts and activities supported by helping them to tie up with research institutions. Thirdly, biochemistry and biotechnology of wild economic plants be pursued for future need-base commercial access and a chain of botanical gardens be established at different altitudes to conserve and multiply the germplasm of rare and endangered species.

Some of the important medicinal plants of the state are *Andrographis paniculata*, *Cinchona* spp., *Phyllanthus emblica*, *Bacopa monnieri*, *Terminalia arjuna*, *Ficus religiosa*, *Saraca asoca*, *Croton tiglium*, *Cissus quadrangularis*, *Colocasia* spp., *Spondias pinnata*, *Streblus asper*, *Ocimum sanctum*, *Cynodon dactylon*, *Aegle marmelos*,


Xanthoxylum oxifolium, Rubus alceifolius, Meyna spinosa, Ficus benghalensis, Withania somnifera etc. All these plants need scientific validation as well as detailed documentation including molecular parameters.

*Spondias pinnata* Kurz (Syn. *Spondias mangifera* ; Assamese name - amara) is an important medicinal plant belonging to anacardiaceae family, commonly found in Assam and the adjoining states which has many ethno-medicinal uses. Roots are used in regulating menstruation in womanfolk. The aromatic astringent and refrigerant bark is used in dysentery, diarrhoea, vomiting and muscular rheumatism. Leaves are also aromatic, astringent and used in dysentery; juice extracted from leaves is recommended for local application in otalgia. The ripe fruits are sweet, astringent, cooling, emollient, tonic, constipating and antiscorbutic as well as useful in bilious dyspepsia, diarrhoea, general debility and vitiated conditions of tridosa (Warrier *et al.*, 1996).

*Streblus asper* Lour (Assamese name – Soura or Siora) is a small evergreen tree of moraceae family, abundantly found throughout the state and adjoining states. This plant is known for its medicinal properties. The decoction of its bark is consumed in fever, dysentery and diarrhoea whereas the root is known to possess antiseptic and astringent properties. The decoction of the leaves of the plant is prescribed in the treatment of high blood pressure, and also for easy painless and quick delivery during labour. The boiled juicy extract of the stem is used against common intestinal worms for large animals. The ability of the tree foliage to remain green during the dry season makes them a potential source of protein and energy supplement to both domestic and wild animals. To avoid the problem of gastrointestinal worms and poor nutrition in grazing animals, fresh leaves of *Streblus asper* is fed at least for seven days, along with other
fodder species. Leaves of the plant are used by traditional fisherman of the state as an antidote against ‘flesh decaying disease’ of cultivated fishes. Moreover, branch segments of the plant are used as toothbrush and are believed to contain compounds effective against tooth decay. It is reported to be a favoured food of rhinoceros. The plant is distributed in the entire North Eastern region; however, the population is dwindling rapidly with the possibility of being extinct in near future.

1.11. Documentation and identification of medicinal plants

Plants have been used in traditional medicine for several thousand years. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use about 2,500 plant species and 100 of them serve as regular sources of medicine (Mutthu et al., 2006). During the last few decades, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Documentation of the indigenous knowledge through ethnobotanical studies and molecular characterization is important for the conservation and utilization of biological resources. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases. Due to poor communication, poverty, ignorance and unavailability of modern health care facilities; most of the people especially tribal and rural mass in the North Eastern Region are still forced to practice traditional medicines for their common ailments. Most of these people form the poorest link in the trade of medicinal plants. A vast knowledge on the use of plants against different illnesses might have accumulated in areas where the use of plants is still of great importance. Traditional knowledge on
medicinal plants and their use by indigenous cultures are not only useful in the
conservation of traditions and biodiversity but also for the community healthcare and
drug development in the present and future. Apart from the tribal groups, many other
forest dwellers and rural people also possess unique knowledge about plants. Due to lack
of interest among the younger generation as well as their tendency to migrate to cities for
lucrative jobs, this great wealth of knowledge is declining. Right from its beginning, the
documentation of traditional knowledge especially on the medicinal uses of plants, has
provided many important drugs of modern day. But, information on the use of plants for
medicine and molecular parameters present in them from this area of the country is rare.
Thus, many important leads to drug discovery may be lost in the absence of proper
documentation and identification of the medicinal plants.

1.12. Active compounds in medicinal plants

Plants have an almost limitless ability to synthesize aromatic substances, most of
which are phenols or their oxygen-substituted derivatives. They are usually secondary
metabolites, of which at least 12,000 have been isolated, which is less than 10% of the
total (Schultes, 1978). In many cases, aromatic substances serve as the component of
plant defense mechanism against microorganisms, insects and herbivores. Some, such
compounds like terpenoids, give plants their aroma; others (quinones and tannins) are
responsible for plant pigment. Many compounds are responsible for plant flavor, such as
the terpenoid capsaicin from chili, pepper, and some other herbs and spices used by
human beings to season food, yield useful medicinal compounds (Cowan, 1999). Medicinal plants have attracted considerable research attention due to their prospect to
use as antimicrobial agents. The natural products derived from medicinal plants have
proven to be the abundant source of biologically active compounds, many of which have been the basis for the development of new pharmaceutical molecules. With respect to infectious diseases, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics, has led to the renewed interest in the discovery of novel antiinfective compounds from plants and such other sources. As there are approximately 5,000,000 plant species occurring worldwide, of which only 1.0% (5,000) has been phytochemically investigated; thus there is great potential for discovering novel bioactive compounds (Palombo, 2006). However, according to the World Conservation Monitoring Centre under the United Nations Environment Programme, the current extinction rates of plants and animals mean that the world is losing one major drug every two years (Groombridge and Jenkins, 2002). Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. One of such resources is the folk medicine. Systematic screening of them may result in the discovery of novel effective compounds.

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. Contrary to the synthetic drugs, antimicrobial compounds of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Janovská et al., 2003).

1.13. Genome size

Genome size of an organism is the amount of nuclear DNA in its unreplicated gametic nucleus, irrespective of the ploidy level or taxon (Singh, 2003). It is measured by
weight or number of base pairs where 1 picogram (pg) equals to 978 megabases (Mb). Hinegardner (1976) described genome size as an important biodiversity character with fundamental significance and wide range of modern biological uses. Till date, genome size of only a fraction of plant species are known (Dolezel and Bartos, 2005). Any breeding program requires information on nuclear DNA content or genome size of the particular plant species. Furthermore, geo-botanical studies may use genome size as an additional parameter for the interpretation of species-specific phenology and the composition of plant communities (Lysak et al., 2000). Interest in the origin, extent and significance of genome size variation has increased greatly in the last decade. Nuclear DNA amount and genome size (C-value) are important biodiversity characters, whose study provides a strong unifying element in biology with practical and predictive uses. Variation in DNA amount between species begins with changes within species, yet intraspecific variation remains one of the most controversial topics in the study of plant genome size. Dolezel and Bartos (2005) observed that previous estimations of DNA peak ratios obtained in four different laboratories for otherwise identical species pairs differed between 1.8 and 15.6%. They opined that absolute DNA amounts of a given plant species determined in different laboratories should be compared with caution and in no way should the differences be interpreted in terms of intraspecific variation in genome size. It is clear that genome size impacts on other areas of research and its knowledge can be important when framing questions or planning research. DNA fingerprinting and quantitative genetics are two such examples where knowledge of genome size may be important. Microsatellites are used widely for DNA fingerprinting in population genetic studies analysing population structure, gene flow, genetic diversity etc and yet their
successful analysis has been shown in part to be determined by genome size. There exists a highly significant positive correlation between genome size and the successful amplification of microsatellites (Garner, 2002). The use of a related DNA fingerprinting technique, amplified fragment length polymorphisms (AFLPs), is similarly affected by the genome size. The knowledge of genome size and ploidy level generated on the basis of AFLP analyses on plant species ranging in C-value from 0.2 to 32.25 pg are important for determining what protocol is most likely to yield informative data for population genetic analyses (Fay et al., 2005). Genome size has now been recognized to be potentially important in the field of quantitative genetics, which aims to analyse and understand the genetic basis of characters showing continuous variation. A simple and less expensive method on genome size determination was developed by Konwar et al. (2007)

Objectives

*Spondias pinnata* Kurz. and *Streblus asper* Lour are two of the important medicinal plants of the entire North East India including the state of Assam. Proper phytochemical analysis and documentation of knowledge on these two plant species may lead to their effective use, conservation and improvement.

On the basis of the above facts, the present investigation has been proposed with the following objectives:

1. To extract and identify major bioactive compounds present in *Spondias pinnata* Kurz. (*Amara*) and *Streblus asper* Lour. (*Soura*).
2. To assess the antimicrobial activity of the bioactive compounds under *in vitro* condition and their structural elucidation.
3. To determine the genome size of the plants.